Active phytochemicals present in the Guava Tree (*Psidium Guajava*) leaf Extracts that grow in Uganda.

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Abstract Background: The Guava tree is small in the myrtle family (*Myrtaceae)*, native to Mexico, Central America, the Caribbean, and northern South America. It contains a high content of organic and inorganic compounds like secondary metabolites such as antioxidants, polyphenols, antiviral compounds, and anti-inflammatory compounds. Such chemicals are produced by plants through primary or secondary metabolism whereas Guava products have been used as a source of medicine and while they have proved to work effectively, little is known about the composition of such plant origin products from Uganda.

Methodology: The maceration method of extraction was used to obtain the guava extract. The leaves were crushed into a powder using a blender and 20g of the powder was added into differently labeled beakers containing 100mls of distilled water and 100mls of increasing concentration of methanol from 30%, 50%, and 70%. The analysis involved Saponins, Phenols and Tannins, Terpenoids, Flavonoids, and Glycosides, and Laboratory tests are done included the Ferric Chloride test, Shinoda test, Salkowski test, Concentrate H2SO4 test, and Foam test.

Results and discussion: Phytochemicals (Saponins, Phenols and Tannins, Terpenoids, Flavonoids, and Glycosides) are present in the leaves of the guava tree (Psidium guajava) that grows in Uganda. *Psidium guajava* extract obtained using distilled water contained all the phytochemicals tested apart from terpenoids which showed a negative test result. Methanol of increasing concentrations can also be used to extract phytochemicals from the leaves.

Conclusions and recommendations: It's now evident that the Guava tree that grows in Uganda contains phytochemicals (secondary metabolites). Water and methanol of 30%, 50%, and 70% can be used for extraction. Future detailed studies in Africa should focus on the purification of these active secondary metabolites and also determine their concentration. Other parts of the plant such as its bark, roots, and fruits need to be studied.

1 Introduction:

The Guava tree is a common tropical fruit cultivated in many tropical and subtropical regions (*Psidium guajava* (common guava, lemon guava) is a small tree in the myrtle family (*Myrtaceae*), native to Mexico, Central America, the Caribbean, and northern South America Morton, et al., (2015) The most frequently eaten species, and the one often simply referred to as "the guava", is the apple guava (*Psidium guajava)*.

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According to Naseer *et al.*, (2018), Guava contains a high content of organic and inorganic compounds like secondary metabolites like antioxidants, polyphenols, antiviral compounds, anti-inflammatory compounds some of which have shown to assist in curing cancerous cells and prevent skin aging before time. Guava leaves contain many compounds that act as fungistatic and bacteriostatic agents. These contain a high content of important antioxidants and have a radio-protective ability.

Phytochemicals are chemicals produced by plants through primary or secondary metabolism (Molyneux *et al.*, 2007 and Harborne *et al.,* 1999). Phytochemicals play a role in plant growth or defense against competitors, pathogens, or predators (Molyneux *et al*., 2007) and due to this ability, they have attracted a lot of attention from researchers.

In Africa, plant origin products have been used as a source of medicine and while they have proved to work effectively, little is known about the composition of such plant origin products. Early studies outside Uganda have studied the guava tree and have confirmed the presence of phytochemicals. This is, however, missing in Uganda. The guava tree is considered one of the valuable trees in traditional medicine and despite this fact, little is documented about the active phytochemicals that are present in the local species and the best way to extract them out of the leaves. This study aimed to detect the presence of such phytochemicals in the guava trees that grow in Uganda and the different extraction methods that can be used.

2 Methodology:

3 Materials and Methods

Data Collection Methods

Instruments:

The tools used in the study included analytical scale, blender, sieves, desiccators, Petridis, stir bar, pipettes, flask filter paper, aluminum foil, vortex, incubator, centrifuge, and platform shaker, sieve, stop the clock, cock borer, and labeling pencil.

Material:

Materials used in the extraction included the guava leaves, distilled water, technical ethanol of 30%, 50%, and 70%, sodium carbonate(Na2CO3) 7%, iron (iii) chloride(FeCl3) 1%, magnesium ribbon fragments, concentrated hydrochloric acid, chloroform, concentrated Sulphuric acid, Glacial acetic acid.

Preparation of the plant extract

The guava leaves were collected from a plantation near the zika forest and samples were put in a bag and transferred to the university laboratory where it was identified. The guava leaves were then washed using tap water, left to dry, and then weighted, its average weights were measured and recorded. The average wet mass was 2.5g for each leaf. The guava leaves were left to dry at 29 °C for 7 days and the average weight was 0.83g and this was captured as dry mass.

Extraction method

The maceration method of extraction was used to obtain the guava extract. The leaves were crushed into a powder using a blender and 20g of the powder was added into differently labeled beakers containing 100mls of distilled water and 100mls of increasing concentration of methanol from 30%, 50%, and 70%.

The powder was then mixed with the solvents using a vortex for 2 minutes and the flasks were covered with aluminum foil and left to stand at the working bench for 3days at 29 °C. The flasks were then shaken vigorously after the 3 days of soaking in a solvent, then, the mixtures were transferred to 4 mL tubes and centrifuged for 10 min at 4,000 rpm at 29°C. The supernatant was collected and stored at 4°C until use.

Detection of the active ingredients

The analysis involved Saponins, Phenols, and Tannins, Terpenoids, Flavonoids, and Glycosides.

Phenols and tannins (Ferric Chloride Test).

The crude extract was boiled with 5 ml of 45% solution of ethanol for 5 minutes. Each of the mixtures is cooled and filtered then used to do the test.

To the 2mls of extract added in a test tube, 2mls of 2% solution of Iron(III) chloride (FeCl3) was added.

Flavonoids (Shinoda Test).

Table 1. shows the results obtained from the detection of the active phytochemicals present in the guava extract.

	Water extract	30% methanol	50% methanol	70% methanol
Saponins	**Positive**	**Positive**	**Positive**	**Positive**
Phenols and Tannins	**Positive**	**Positive**	**Positive**	**Positive**
Terpenoids	**Negative**	**Positive**	**Positive**	**Positive**
Flavonoids	**Positive**	**Positive**	**Positive**	**Positive**
glycosides	**Positive**	**Positive**	**Positive**	**Positive**

To the 2mls of extracts in the test tube, magnesium ribbon fragments were added plus concentrated hydrochloric acid droop wise.

Terpenoids (Salkowski test).

2mls of extract in the test tube were mixed with 2mls of chloroform then 2mls of concentrated Sulphuric acid was added and shaken gently.

Glycoside (Concentrate H2SO4Test).

To 2mls of extract in the test tube, 2mls of glacial acetic acid plus 2 droops of 2% iron (III) chloride plus 2mls of concentrated. Sulphuric acid was added.

Saponins (Foam Test).

2mls of each extract was added to the test tube and vigorously shaken.

4 Results and discussion

Analysis of the phytochemicals present

The table below shows the results of the quantitative tests done to detect the presence of the active phytochemicals present using the different extraction solvents.

Phenols and tannins results (Ferric Chloride Test)

Observation- the formation of a blue-green or black coloration indicated the presence of phenols and tannins.

Figure 1. showing the blue green or black coloration indicating presence of phenols and tannins

Flavonoids test results:

Observation- the formation of a pink coloration indicated the presence of flavonoids.

Terpenoids test results

Figure 2. shows the orange, red, pink coloration indicating presence of flavonoids.

Observation- the formation of a reddish brown coloration of the interphase indicated the presence of terpenoids.

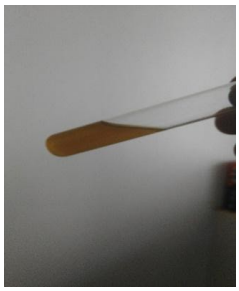

Figure 3. shows a reddish brown coloration

Glycosides test results

Observation- the formation of a brown ring indicated the presence of glycosides.

Figure 4. shows the brown rings an indication of glycosides

Saponins test results

Observation The formation of stable foam was an indicator that Saponins were present.

Figure 5. showing the formation of a stable foam

5 Discussion

Findings of similar investigations have reported that there are active phytochemicals in the different plant parts. According to a study by Senguttuvan *et al.*,(2014) showed that Glycosides, Flavonoids, Phenols, Tannins, Saponins are highly present in the extract of leaves obtained from *H. radicata* and Terpenoids are present in moderate amounts when methanol and water are used as the extraction solvents. The results of the current study also have shown that the same phytochemicals are present in the leaves of the guava tree (*Psidium guajava*). Senguttuvanm *et al.,* (2014) results showed that water as the extraction medium did not extract Glycosides, Phenols, Tannins, and Saponins from *H. radicata* leaves. However, the results from the present study show that the *Psidium guajava* extract obtained using distilled water contained all the phytochemicals tested apart from terpenoids which showed a negative test result when water was used for extraction. The observations made in this study (formation of a stable foam to confirm Saponins, the formation of a pink coloration for Flavonoids, and the formation of a brown ring indicating the presence of Glycosides using Concentrate H2SO4 test) were similar to ones reported by Rahman *et al.,* (2017) in the study were Phytochemical Screening was done on Crude Plant Extracts from *Ephedra intermedia*. The formation of A transient greenish to black color that indicated the presence of Tannins in this study was in agreement with a study by Sheel *et al*., (2014) where Phytochemical Screening of Methanolic Extract of Clerodendron infortunatum was done using the Ferric Chloride Test. The pink coloration as an indication of flavonoids in this study agrees with the results reported by Sumalatha *et al.,* (2012) when Isolating Flavonoids of Tephrosia purpurea when the Shinoda Test was used.

6 Conclusion and recommendations

It's now evident that the Guava tree (*Psidium guajava*) that grows in Uganda contains phytochemicals (secondary metabolites). The solvents used in the extraction such as water and methanol of 30%, 50%, and 70% can be used to extract the active phytochemicals. Future detailed studies in Africa should focus on the purification of these active secondary metabolites and also determine their concentration. This study has focused on the leaf of a guava tree, this presents other parts of the plant such as its bark, the roots, and the fruits for more studies.

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